Procedures for Collecting Surface Environmental Samples for Culturing *Bacillus anthracis*

Preface

The decision to collect environmental samples for culturing *Bacillus anthracis* should be made by medical, environmental, and industrial hygiene professionals familiar with the organism and with the environmental sampling methodologies described in this document. This decision should be based on the nature and location of the suspected contamination, the medical diagnoses and opinions, the potential for the contaminant to migrate, and the activity for which the facility is used, following a pre-planned sampling strategy. Representatives from local, state, and federal agencies should be consulted during the decision-making process.

Environmental sampling can be used to help determine the extent and degree of contamination, to support decisions regarding the need for cleanup, and to provide guidance regarding when cleanup is adequate to permit re-entry into an area. The use of experienced investigators to conduct the environmental sampling will provide the best probability of locating and identifying *B. anthracis* spores if present.

Currently, no environmental exposure standards exist for *B. anthracis* spores. Investigators who review and interpret the results of environmental sampling for such spores must consider these uncertainties and use professional judgment in interpreting any positive or negative findings.

Before sampling is begun, the building's engineer should be consulted to determine airflow patterns and the design of the heating, ventilating, and air-conditioning system(s). Since most building ventilation systems recirculate air to other locations in the building, the ventilation system serving the contaminated area should be shut off to prevent further airborne spread of any *B. anthracis*. Depending on the size of the area involved, the types of surfaces potentially contaminated, and the extent of contamination, it may be necessary to isolate and control access to the contaminated area to prevent the spread of contamination through the movement of people or equipment:

- If the contaminated area is small, discrete, and only lightly contaminated, cordoning off the area may provide adequate protection.
- If the contaminated area is large, the affected area should be sealed off using an interim dust barrier made from impervious lightweight plastic (e.g., 6 mil polypropylene) sheeting. Tight seals should be maintained at the full perimeter of temporary walls and sealed by tape at ceiling height in the same way that areas are sealed off for asbestos abatement or dust control during building renovation. Air vents in the area should also be sealed with plastic sheeting and tape to control the risk of dust dispersal and recirculation.

The sampling method and number of samples collected will be influenced by the nature, circumstances, and setting of the potential contamination. The methods used may include bulk or surface sampling strategies. Since the extraction efficiency of spores from the various building materials is not known, the results of bulk or surface sample analyses are

qualitative. A sufficient number of samples must be taken to increase the probability that the sampling is representative. Obtaining samples from additional locations may provide more specific information on the source of the contamination. For each sample collected, the usual, non-forensic chain-of-custody procedures should be followed and documented as designated by the local state health laboratory reporting requirements. Taking photographs of the location where the samples are collected is often helpful.

The first priority should be to collect samples in locations that are near suspected release source(s). Samples should be collected by moving inward in concentric circles toward the suspected release source, following the path over which spores may have dispersed. If the aerosol containing *B. anthracis* spores has an aerodynamic size of less than 10 microns, the particles will remain suspended in the air for extended periods of time. In such cases, the spores can quickly spread throughout an air space and into adjacent areas. Spores can also be carried if they attach to clothing, shoes, or other objects; thus, more distant sampling may be needed. (Personnel who enter the contaminated area must follow a safety and infection control plan developed for the particular site).

Bulk sampling
Bulk samples can help investigators characterize the presence of contamination on building materials such as carpets, office equipment, and supplies. However, because extracting spores from bulk samples can pose exposure concerns for laboratory personnel, appropriate precautions (such as double-bagging of samples) should be taken.

Surface sampling
Surface samples are collected by wiping non-porous surfaces with an absorptive medium from which spores can be extracted in the laboratory. The absorptive media, wetting agent, and bags used to transport samples should be selected with input from the laboratory personnel who will be analyzing the samples so that collection procedures will be compatible with the laboratory's analytical procedures. There are several absorptive media available, but noncotton swabs are preferred. These swabs must be sterile and used with a sterile wetting agent such as sterile water, a sterile saline solution, or a sterile phosphate-buffer solution.

Samples collected by vacuuming
Although collecting samples by vacuuming offers the advantages of covering large surfaces and collecting material from porous areas such as carpets, only high-efficiency particulate air (HEPA) vacuum cleaners must be used. Conventional home or industrial vacuum cleaners should not be used for sample collection because these vacuum cleaners will further disperse spores. A hose or diffuser can be retrofitted to the vacuum cleaner exhaust so that the HEPA-filtered exhaust can be vented outside the contaminated zone to prevent reaerosolization of spores within the contaminated area. There are several methods for collecting vacuum samples. One option is to connect a filtering Alsock® (dust collection trap manufactured by Healthy Home Air or equivalent)* to the inlet nozzle of a HEPA vacuum cleaner. A second option is to collect a sample on a 37-millimeter (mm) diameter mixed cellulose ester (MCE) filter contained in a plastic sampling cassette. The analytical results from this type of sampling are qualitative.

Finally, when selecting sampling equipment, consideration should be given to whether and how it can be decontaminated.

Collecting Bulk Samples

For most laboratories, bulk samples are not acceptable. Therefore, the receiving laboratory should be contacted before bulk samples are collected to determine whether such samples will be accepted. Bulk samples may include items such as sections of carpet, office equipment, supplies, or vials of dust.

1. Don sterile, non-powdered nitrile or vinyl examination gloves over the gloves that are part of standard PPE and clothing.
2. Collect and bag the item; seal the bag.
3. Clean the outside of the sealed bag with a 0.5-0.6% (5,000-6,000 ppm) sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains approximately 5.25-6% (52,500-60,000 ppm) sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH range of ~6-8. Clorox® bleach* diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
4. Place the cleaned, sealed bag in another unused, self-sealing bag, and prepare for shipping according to CDC guidelines (http://www.cdc.gov/od/ohs/biosfty/shipregs.htm or http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf).
5. Record the following items:
    - Measured size of the area sampled
    - Type of sample
    - Time and date of sample
    - Name of person collecting sample

To collect another sample, change gloves to prevent cross-contamination and repeat steps 1-5.

6. Submit the samples to the laboratory for culture.
7. Transport samples to the laboratory at ambient temperature.
8. Maintain appropriate chain-of-custody documentation and procedure.

Collecting Sterile Swab Samples

The following steps are used to collect samples for laboratory culture from small non-porous surfaces or objects.

1. Don sterile, non-powdered nitrile or vinyl examination gloves over the gloves that are part of standard PPE and clothing.
2. Remove a sterile, non-cotton swab from the package.
3. Moisten the swab with 100-200µl (or 1-2 drops) of a sterile water, sterile saline, or sterile phosphate-buffered saline (PBS) solution. This can be done by using a disposable Pasteur pipette and aseptic technique. Note: Check with the laboratory that will do the analysis to determine which type of swab or solution is preferred.
4. Wipe the surface. Recommended wipe area is ≤100 cm^2. Avoid letting the swab dry completely.
5. Place the sampled swab into a sterile conical vial, and cap the vial.
6. Record the following items:
    - Measured size of the area sampled
    - Type of sample
    - Time and date of sample
    - Name of person collecting sample
7. Label the vial, and place it in a self-sealing bag (such as a Ziploc® bag or Whirlpak®).*
8. Clean the outside of the sealed bag with a 0.5-0.6% (5,000-6,000 ppm) sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains about 5.25-6% (52,500-60,000 ppm) sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH.range of ~6-8. Clorox® bleach diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
9. Place the cleaned, sealed bag in another unused similar bag.

To collect another sample, change gloves to prevent cross-contamination and repeat steps 1-9.

10. Prepare samples for shipping according to CDC guidelines (http://www.cdc.gov/od/ohs/biosfty/shipregs.htm or http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf) and submit the samples to the laboratory for analysis.
11. Transport samples to the laboratory at ambient temperature.
12. Maintain appropriate chain-of-custody documentation and procedure.

LRN Level A Protocol for Rule-Out Testing of *Bacillus anthracis*

1. Process low-risk (non-powder) environmental samples taken and transported according to above procedure in a CLIA-certified laboratory using biosafety level (BSL) 2 facilities and BSL-3 safety practices.
2. Follow standardized Laboratory Response Network (LRN) Level A testing protocol (http://www.asmusa.org/pcsrc/ban.asm.la.cp.102401f.pdf) with modification for elution and plating as follows (per LRN Level B):
    a. Place each sample swab in 3 ml of sample-processing solution (0.3% Tween in PBS).
    b. Vortex for approximately 1 minute.
    c. Transfer 1.5 ml of buffer to a tube labeled "C." Label the remainder of the sample "A."
    d. Heat-shock the "C" tube in the 65°C water bath for 10 minutes.
    e. Inoculate the "A" and "C" samples onto 3 blood agar plates each using 0.1 ml inoculation volumes.
    f. Streak the plates for isolation.
    g. Incubate the plates at 35-37°C for 18-24 hours and examine for suspicious colonies. Identify suspicious colonies using the LRN Level A *Bacillus anthracis* methods referred to above.

    OR

    a. Remove the swab from the transport container and place into 1.5 ml of sterile saline or a nutrient broth such as trypticase soy broth, brain heart infusion broth, or equivalent. Vigorously twist the swab, and recap the tube.
    b. Leave the swab in the tube. Place the broth suspension into a 65°C water bath for 30 minutes.
    c. Plate 0.1-0.2 ml of broth on 5% sheep blood agar plate and incubate at 35-37°C for 18-24 hours. Many *B. anthracis* will have visible growth in 12-18 hours; observe for characteristics of *B. anthracis*.

***All culture isolates that cannot be ruled out and are therefore presumptively positive should be referred to an LRN State Public Health Laboratory for confirmatory testing by the LRN Level B protocol and standardized algorithm for identification of* Bacillus anthracis.**

Collecting Samples with a HEPA Vacuum Cleaner

The following steps should be used to collect samples for laboratory culture from large porous or non-porous surfaces such as carpeting, ceiling tiles, ventilation systems, and papers.

1. Don sterile non-powdered nitrile or vinyl examination gloves over the gloves that are part of the standard PPE and clothing.
2. Insert a cone-shaped filtering Alsock® (dust collection trap manufactured by Healthy Home Air or equivalent)* into the vacuum cleaner nozzle.
3. Fold the plastic sleeve over the outside of the nozzle, and secure it with tape or an elastic band.
4. HEPA-vacuum the surface. Note: 1-2 tablespoons of vacuumed debris are needed. Technique: Make one pass of the entire sampling area at a slow rate (12 inches per 5 seconds).
5. Record the following items:
    - Measured size of the area sampled
    - Type of sample
    - Time and date of sample
    - Name of person collecting sample
6. After collecting the sample, remove the tape or elastic band and discard these items as contaminated waste.
7. Remove the cone-shaped dust collection trap, and place it in a self-sealing bag (such as a Ziploc® bag or Whirlpak®),* or roll the filter and place it in a sterile conical vial.
8. Place the sample in a clean self-sealing bag and label it.
9. Clean the outside of the sealed bag with a 0.5-0.6% (5,000-6,000 ppm) sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains about 5.25-6% (52,500-60,000 ppm) sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH range of ~6-8. Clorox® bleach diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
10. Place the cleaned sealed bag in another unused self-sealing bag.

To collect another sample, change gloves and clean the nozzle with the bleach solution followed by alcohol to prevent cross-contamination, and repeat steps 1-10.

11. Prepare samples for shipping according to CDC guidelines (http://www.cdc.gov/od/ohs/biosfty/shipregs.htm or http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf) and submit the samples to the laboratory for analysis.
12. Transport samples to the laboratory at ambient temperature.
13. Maintain appropriate chain-of-custody documentation and procedure.

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*Use of trade names is for identification and information only and does not constitute an endorsement by the Department of Health and Human Services or the Centers for Disease Control and Prevention.